(a) probing said region of interest to determine target species density within said region of interest by detecting fluorescence emitted by a quantum dot attached to one or more molecules of said target species bound to an affinity moiety for said target species immobilized on said substrate;

(b) comparing said density to a predetermined density cutoff value above which ensemble counting is used and below which single target counting is used, thereby determining whether said target species is quantifiable by target counting or ensemble counting.

REMARKS

The present invention

The present invention provides methods of counting a single copy of a target species immobilized on a substrate by detecting fluorescence emitted by a quantum dot attached to the single copy and resolving the fluorescence emitted by the attached quantum dot from fluorescence arising from other sources, e.g., a quantum dot not attached to the single copy.

Known assays for detecting target species labeled with quantum dots use ensemble counting. Ensemble counting refers to the detection of signal from a plurality of labeled targets in a detection field in the form of average emission intensity over the area of the detection field. Rather than using ensemble counting to detect emission from a quantum dot or multiple quantum dots attached to *multiple copies* of a target species and quantifying average emission data from a *population* of target species, the present application teaches *single target* counting to detect emission from a quantum dot or multiple quantum dots attached to a *single copy* of a target species and resolving that emission from emissions arising from other sources, thereby counting *single copies* of a target species. In ensemble regimes, sample concentration is proportional to average emission intensity. Accordingly, detection assays using ensemble counting suffer from

limited sensitivity, specificity and dynamic range. Because the present invention provides methods of counting *single copies* of a target species by resolving emissions from single copies of the target species, the present invention provides a novel and non-obvious assay with great specificity, sensitivity and dynamic range.

Status of the claims

With this amendment, claims 1-25 and 28-39 are pending in the present application and under examination. Claims 1, 4, 10, 22, 25, 28-33, and 37-38 are amended. Claims 26 and 27 are canceled without prejudice to subsequent revival. Appendix A provides the version with markings to show change to the amended claims. Appendix B shows all pending claims currently under examination.

Claims 1, 4, 10, 22, 25, 28-33, and 37-38 have been amended to more distinctly claim the present invention and in accordance with the Examiner's suggestions. These amendments add no new matter. Support for them can be found, e.g., in the claims as filed and on page 14, lines 29-34.

For convenience, the Examiner's rejections are addressed in the order in which they were presented in the February 1, 2002 Office Action.

Claim Objections

Claim 10 is objected to for containing two commas. In response, Applicants have amended claim 10 to remove one comma. Accordingly, Applicants request that the objection be withdrawn.

Claim 32 is objected to for missing the word "and". In response, Applicants have amended claim 32 to add the word "and". Accordingly, Applicants request that the objection be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph, indefiniteness

"said first quantum dot"

Claim 4-8 are rejected as allegedly indefinite for claim 4 reciting the phrase "said first quantum dot," without proper antecedent basis. In response, in accordance with the Examiner's suggestion, Applicants have, inserted the phrase "first quantum dot and" before "second quantum dot". Accordingly, Applicants respectfully request that the rejection be withdrawn.

"is correlated with"

Claim 22 is rejected as allegedly indefinite for reciting the phrase "is correlated with". In response, in accordance with the Examiner's suggestion, Applicants have replaced "is correlated with" with "identifies". Accordingly, Applicants respectfully request that the rejection be withdrawn.

"such that"

Claim 31 is rejected as allegedly indefinite for reciting the phrase "such that". In response, in accordance with the Examiner's suggestion, Applicants have replaced "such that" with "whereby". Accordingly, Applicants respectfully request that the rejection be withdrawn.

"omitting essential steps"

Claim 32-36, and 37 are rejected as allegedly indefinite for omitting essential method steps of target detection. In response, in accordance with the Examiner's suggestion, Applicants have amended claim 32 and 37 to recite method steps of target detection. Accordingly, Applicants respectfully request that the rejection be withdrawn.

Claims 38-39 are rejected as allegedly indefinite for omitting steps determining whether a target species is quantifiable. In response, in accordance with the Examiner's suggestion, Applicants have amended claim 32 and 37 to recite method steps

determining whether a target species is quantifiable. Accordingly, Applicants respectfully request that the rejection be withdrawn.

Rejections under 35 U.S.C. § 101

Claim 25-28 are rejected under 35 U.S.C. § 101 as directed to non-statutory subject matter. In response, Applicants have canceled claims 26 and 27 and have amended claims 25 and 28 to refer to a computer readable medium encoded with a data set or database. MPEP 2106 IV.B.1(a) states that "a claimed computer-readable medium encoded with a data structure defines structural and functional interrelationships between the data structure and the computer software and hardware components which permit the data structure's functionality to be realized, and is thus statutory". Accordingly, the claims as amended refer to statutory subject matter. Applicants therefore respectfully request that the rejection be withdrawn.

Rejections under 35 U.S.C. § 102(e)

Claims 1-7, 9-18, and 29 are rejected as allegedly being anticipated by Bawendi *et al.* (U.S. Patent No. 6,306,610 B1). The Examiner alleges that Bawendi *et al.* anticipates the claimed invention because the Bawendi *et al.* patent allegedly discloses methods of detecting a single copy of a target species by detecting fluorescence emitted from a quantum dot attached to a single copy of a target species. To the extent that the rejection applies to the claims as amended, Applicants respectfully traverse.

As the Examiner is well aware, for a rejection under § 102(e) to be properly founded, a single prior art reference must disclose, either expressly or inherently, each and every element of the claimed invention. *See, e.g., Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987); and *Verdegaal Bros. V. Union Oil Co. Of California*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). In *Scripps Clinic & Research Found. v. Genetech, Inc.*, 18 USPQ2d 1001 (Fed. Cir. 1991), the Federal Circuit held that:

> Invalidity for anticipation requires that all of the elements and limitations of the claim are found with a single prior art reference. . . .There must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention. *Id.* at 1010.

Anticipation can be found, therefore, only when a cited reference discloses all of the elements, features or limitations of the presently claimed invention.

The rejection cites Bawendi *et al.* as the basis for the § 102(e) rejection. Applicants respectfully submit that the Bawendi reference does not disclose every element of the presently claimed invention and, thus, cannot form the basis for a § 102(e) rejection. Namely, the cited reference does not expressly or inherently disclose that the fluorescence emission from the quantum dot or dots attached to a single copy of a target species be resolved from fluorescence arising from quantum dot or dots not attached to the single copy of the target species.

The Bawendi *et al.* patent discloses a method of detecting targets labeled with quantum dots. The Bawendi *et al.* patent, however, does not disclose a method of counting a *single copy* of a target species labeled with a quantum dot by resolving the fluorescence from a quantum dot or quantum dots attached to a single copy of a target species from fluorescence arising other sources, e.g., a quantum dot not attached to said single copy. In particular, the Bawendi *et al.* patent does not expressly or inherently set forth the element that the fluorescence emitted by a quantum dot or quantum dots attached to a single copy of a target species is resolved from fluorescence arising from other sources, e.g., a quantum dot not attached to the single copy. As Bawendi *et al.* fails to teach every element of the claimed invention, the Bawendi *et al.* patent cannot be said to anticipate claims 1-7, 9-18, and 29.

In the Bawendi *et al* patent, fluorescence emitted from multiple quantum dot or quantum dots attached to a multiple target nucleic acid species is detected and quantified by previously known methods. Namely, emission from a *population* of species is detected, quantified, and used to determine location and amount of target species present in the sample. In contrast, claim 1 of the present application now

expressly recites the method of counting a single copy of a target species by resolving the fluorescence emitted by a quantum dot or quantum dots attached to a single copy of a target species from fluorescence arising from other sources. The Bawendi patent neither teaches nor suggests resolving the fluorescence emitted from a quantum dot attached to a single copy of a target species. Instead Bawendi uses known methods of detecting a population of quantum dots, e.g., ensemble counting.

For example, in order to understand how the detection methods of Bawendi *et al.* contrast to the counting methods of the present invention, it is useful to envision a series of microarray spots with decreasing concentrations of bound target. In the Bawendi *et al.* method, an array spot is covered with bound target and average emission intensity is dependent on the average density of label across the surface of the array. Sample concentration is proportional to average emission intensity. Accordingly, a target species is detected by measuring total emission intensity and then resolving total emission intensity by determining the density of label across the surface of the area. In contrast, in the present invention, individual bound targets are detected one at a time. Accordingly, fluorescence emitting from a quantum dot(s) attached to a single copy of a target species is viewed separately from fluorescence arising from other sources, e.g., a quantum dot not attached to the single copy of the target species, (*see* Application, pages 15-17 and Figures 1-3).

As each and every element of the present invention is not present in the Bawendi reference, Applicants respectfully request that the Examiner withdraw the anticipation rejection.

Rejections under 35 U.S.C. § 103

The Examiner has rejected claim 8 as allegedly being obvious over Bawendi *et al.* in view of Barbera-Guillem (U.S. Patent No. 6,309,701), claims 19-23 as allegedly being obvious over either Bawendi *et al.* in view of Walt *et al.* (U.S. Patent NO. 6,327,410), claims 24-28 and 32-39 as allegedly being obvious over Bawendi *et al.*

by itself, and claims 30 and 31 as allegedly being obvious over Bawendi *et al.* in view of Empedocles *et al.* (*adv. Mater.* 1999, 11(15):1243-1256).

To the extent that the rejection applies to the claims as amended, Applicants respectfully traverse the rejection. M.P.E.P. § 2143 states the following:

> "[t]o establish a *prima facie* case of obviousness, *three* basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)."

All three elements set forth above must be present in order to establish a *prima facie* case of obviousness. In addition, to avoid the pitfall of hindsight, the Examiner must "identify specifically...the reasons one of ordinary skill in the art would have been motivated to select the references and combine them," *In re Rouffet* 47 USPQ2d 1453, 1459 (Fed. Cir. 1998). Applicants respectfully submit, that each of the required criteria for a p*rima facie* case of obviousness has not been met for the following reasons: 1) there is no suggestion or motivation to modify the references; 2) there is no reasonable expectation of success; and 3) the cited art references do not teach or suggest all the claim limitations.

There is no Suggestion or Motivation to Modify the References

Applicants submit that there is simply no motivation or suggestion provided in the cited references to count a single copy of a target species by *resolving* fluorescence emitted by a quantum dot attached to the single copy from fluorescence arising from quantum dots not attached to the single copy.

As the Examiner is aware, obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992).

Bawendi et al. in view of Barbera-Guillem

As discussed above, Bawendi *et al.* teaches a method of detecting an *ensemble of biological compounds* using quantum dots. In contrast, the present application teaches a method of counting *single copies of biological compounds* using quantum dots. Bawendi's method of detecting biological compounds does not envision the step of resolving fluorescence emitted from a quantum dot attached to a single copy of a target species from fluorescence emitted by quantum dots not attached to the single copy.

In contrast to the Bawendi *et al.* patent, the present application teaches the counting of single copies of target species and methods of resolving spectra emitted from a quantum dot attached to a single copy of a target species from background noise and other quantum dots not attached to the single copy. By counting single copies of target species, the present invention can provide information on the concentration and location of the target species.

Barbera-Guillem does not supply the missing teaching that single copies of target species can be counted by the methods of the present invention. In fact, Barbera-Guillem describes the standard ensemble methods for detecting labeled target species. For example, in column 16, lines 38-43, the Barbera-Guillem patent teaches that after detection, the targets species is quantified by "measuring the *intensity* of the fluorescence signal pattern emitted from the fluorescent microspheres bound to the target species, and relating the *intensity* measured to the amount of species." Relating fluorescence intensity to species amount is in direct contrast to the methods of the present

invention which count single copies of target species instead of measuring average emission intensity.

Accordingly, there is no suggestion or motivation in either the Bawendi *et al.* patent or Barbera-Guillem patent read separately, or together, to use the single target counting methods of the present invention.

Applicants respectfully request that the Examiner withdraw the rejection of claim 8 over Bawendi *et al.* in view of Barbera-Guillem.

2. There is No Reasonable Expectation of Success

In addition, in view of the cited references, one of skill in the art would have had no reasonable expectation of success that single copies of target species could be counted by detecting fluorescence emitted from a quantum dot attached to a single copy of a target species instead of by detecting fluorescence emitted from quantum dots attached to a population of target species, "Both the suggestion and the expectation of success must be found in the prior art, not the Applicants' disclosure." *In re Dow Chem. Co.*, 5 U.S.P.Q.2d 1529, 1532 (Fed. Cir. 1988).

Applicants assert that there is no teaching or suggestion in the cited art to modify the teaching therein to arrive at the presently claimed invention. Bawendi *et al.* discloses a method of detecting an ensemble of biological compounds using quantum dots. Barbera-Guillem also discloses a method of detecting an ensemble of biological compounds using quantum dots. Both references are silent as to the detection of single copies of target species. Neither Bawendi *et al.*, nor Barbara Guillem, therefore, can be said to suggest or motivate the methods of the present invention, e.g., counting a single copy of a target species by resolving fluorescence emitted by a quantum dot attached to the single copy from fluorescence arising from quantum dots not attached to the single copy. Neither of the references, either alone or in combination, teach or suggest counting single copies of target species.

Ensemble methods of detecting target species have been known for some time. However, a skilled person, in view of Bawendi *et al.* or Barbera-Guillem, would

have no expectation of successfully increasing the sensitivity, specificity and dynamic range of target species detection by counting single copies of target species. Applicants therefore respectfully request that the Examiner withdraw the rejection of claim 8 over Bawendi *et al.* in view of Barbera-Guillem.

3. The Cited Art References Do Not Teach All Limitations of the Claims

The prior art references must teach or suggest all the limitations of the claims. *In re Wilson*, 165 U.S.P.Q. 494, 496 (C.C.P.A. 1970). Applicants assert that the prior art references do not teach or suggest all the limitations of the claims and therefore, the obviousness rejection is untenable.

Applicants claim a novel method counting single copies of target species. Under *In re Wilson supra, a prima facie* case of obviousness has not been established as each of the limitations of the claims is not taught or suggested in the cited art references. Neither Bawendi *et al.* nor Barbera-Guillem teach or suggest a method for counting single target species. Specifically, neither reference teaches counting a single copy of a target species by resolving fluorescence emitted by a quantum dot attached to the single copy from fluorescence arising from quantum dots not attached to the single copy.

As the prior art references do not teach every element of the claimed invention, Applicants respectfully request that the Examiner withdraw the rejection of claim 8 over Bawendi *et al.* in view of Barbera-Guillem.

Bawendi et al. in view of Walt et al.

Claims 9-23 were rejected as allegedly being obvious over Bawendi *et al.* in view of Walt *et al.* The Examiner alleges on page 10 of the office action:

> It would have been *prima facie* obvious to one having ordinary skill in the art at the time the claimed invention was made to apply the substrate requiring minimal sample volumes as taught by Walt et al to the substrate in the method of Bawendi et al. and to use a glass slide, capillary, or flow cell for the expected benefit of economy of reagents as taught by Walt et al.

In response, Applicants respectfully traverse the rejection.

Bawendi *et al.* is discussed above. Walt *et al.* teaches a microsphere-based analytic chemistry system in which microspheres or particles carrying bioactive agents may be combined randomly or in ordered fashion and dispersed on a substrate to form an array while maintaining the ability to identify the location of bioactive agents and particles within the array using an optically interrogatable, optical signature encoding scheme.

The Walt patent does not supply the missing teaching that single copies of target species can be counted by the methods of the present invention. In the Walt patent, standard methods of detecting biological compounds are contemplated, e.g., ensemble counting (*see* column 22 and 23). The Walt patent does not envision a detection method that resolves fluorescence emitted from a quantum dot attached to a single copy of a target species from fluorescence emitted by quantum dots not attached to the single copy. Accordingly, there is no teaching, motivation, or suggestion in the Walt patent to detect biological compounds by the methods claimed in the present application.

Furthermore, both the Bawendi *et al.* and Walt patents are silent as to the detection of single copies of target species. Accordingly, after reading the Bawendi *et al.* and Walt patents, one of skill in the art would have no reasonable expectation of success that single copies of target species can be counted by detecting fluorescence emitted from a quantum dot or quantum dots attached to a single copy of the target species. Nor would one of skill in the art expect that such a detection method would be more sensitive and specific than the known methods of detecting target species.

Accordingly, because both Bawendi *et al.* and Walt *et al.*, read alone or together, do not teach, suggest or motivate single copy counting methods for the detection of single copies of labeled target species, or provide a reasonable expectation of success for single copy counting methods, the instant claims are not obvious. As such, Applicants respectfully request that the Examiner withdraw the rejection of claim 9-23 under 35 U.S.C. 103 over Bawendi *et al.* in view of Walt et al.

Bawendi *et al.*

Claims 24-28 and 32-39 were rejected as obvious over Bawendi *et al.* The Examiner states on page 10 of the office action:

> It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to modify the counting of Bawendi et al by combining the ensemble and single target counting using both methods (i.e., to count the target species at a first region using ensemble counting and to count the same target species at a second region using single target counting) to thereby confirm quantity of the target species in a sample and optimize target analysis."

In response, Applicants respectfully traverse the rejection.

Bawendi *et al.* is discussed above. As previously explained, the Bawendi *et al.* patent does not teach that single copies of target species can be counted by the methods of the present invention. The Bawendi *et al.* patent does not envision a detection method that resolves fluorescence emitted from a quantum dot attached to a single copy of a target species from fluorescence emitted by quantum dots not attached to the single copy. Instead, the Bawendi patent discloses standard methods of detecting biological compounds, e.g., ensemble counting.

Furthermore, the Bawendi patent is silent as to the detection of single copies of target species. Accordingly, after reading the Bawendi patent, one of skill in the art would have no reasonable expectation of success that single copies of target species can be counted by detecting fluorescence emitted from a quantum dot or quantum dots attached to a single copy of the target species. Instead, one of skill in the art would have a reasonable expectation of success that emission from a *population* of species can be detected, quantified, and used to determine location and amount of target species present in a sample.

Accordingly, because Bawendi *et al.* does not teach, suggest or motivate single copy counting methods for the detection of single copies of labeled target species and does not provide a reasonable expectation of success for single copy counting

methods, the instant claims are not obvious. Accordingly, Applicants request that the rejection of claims 24-28 and 32-39 over Bawendi *et al.* be withdrawn.

Bawendi *et al.* in view of Empedocles *et al.*

Claims 30 and 31 were rejected as obvious over Bawendi *et al* in view of Empedocles *et al.* The Examiner states on page 10 of the office action:

> It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to apply the teaching of Empedocles et al. to the immobilized complexes of Bawendi et al. and using routine experimentation adjust the placement of the complexes on the substrate such that the resolution region is less than the region having the target complex to thereby eliminate any negative effect resulting from photon coupling between the quantum dots as taught by Empedocles et al for the obvious benefit of optimizing experimental conditions to thereby maximize experimental results.

In response, Applicants respectfully traverse the rejection.

Bawendi *et al.* is discussed above.

The Empedocles *et al.* paper does not supply the missing teaching that single copies of target species can be counted by the methods of the present invention.

Empedocles *et al* does not teach that quantum dots attached to target species in a biological sample can be counted using the methods of the present invention, e.g., counting a single copy of a target species by resolving fluorescence emitted by a quantum dot attached to the single copy from fluorescence arising from quantum dots not attached to the single copy. The Empedocles *et al.* reference teaches single quantum dot spectroscopy and discloses physical phenomena related to single quantum dot spectroscopy. In contrast, the present application teaches methods of counting single copies of biological compounds using quantum dots. The Empedocles *et al* paper does not teach methods of using single target counting methods to detect target analytes in a sample. Accordingly, there is no teaching, motivation, or suggestion in the Empedocles *et al* paper to detect biological compounds by the methods claimed in the present application.

Both the Bawendi *et al* patent and the Empedocles *et al* paper are silent as to the detection of single copies of target species. Accordingly, after reading the Bawendi *et al* patent and Empedocles *et al* paper, one of skill in the art would have no reasonable expectation of success that single copies of target species could be counted by detecting fluorescence emitted from a single copy of a target species.

Accordingly, Applicants respectfully request that the Examiner withdraw the rejection of claims 30 and 31 over Bawendi *et al.* in view of Empedocles *et al.*

OBVIOUSNESS-TYPE DOUBLE PATENTING REJECTION

The Examiner has provisionally rejected the claims under the judicially created doctrine of obviousness-type double patenting over U.S. Patent 6,274,323 B1("'323). To the extent that the rejection applies to the claims as amended, Applicants respectfully traverse.

The determination of obviousness-type double patenting by the PTO is the same as obviousness under 35 U.S.C. § 103. *See, In re Braat,* 19 U.S.P.Q.2d 1289 (Fed. Cir. 1991). Applicants herein have amended the claims to recite the step of resolving fluorescence emitted by a quantum dot attached to a single copy of a target species from fluorescence arising from other sources.

The '323 patent neither teaches nor suggests the step of resolving fluorescence emitted by a quantum dot attached to a single copy of a target species from fluorescence arising from other sources. Instead, it discloses known methods for quantifying emission spectra. Namely, the '323 patent discloses methods for detecting total emission intensity and then resolving total emission intensity by determining the density of label across the surface of the area, e.g., ensemble counting.

Furthermore, there is simply no motivation or suggestion in the '323 patent to solve the problem that the present invention solves, e.g., providing an assay that is more sensitive, specific and has a greater dynamic range than known assays. Nor after reading the '323 patent would there be any reasonable expectation of success that single copies of target species can be counted by the methods of the present application.

Accordingly, Applicants request that the rejection be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

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APPENDIX A
VERSION WITH MARKINGS TO SHOW CHANGE MADE

1. (once amended) A method of [detecting] counting a single copy of a target species immobilized on a substrate, said method comprising:

(i) detecting a single copy of said target species by detecting fluorescence emitted by a quantum dot attached to said single copy, wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate, and

(ii) resolving said fluorescence from said quantum dot attached to said single copy from fluorescence arising from a quantum dot not attached to said single copy, thereby counting said single copy.

4. (once amended) The method according to claim 1, wherein said target species has a first quantum dot and a second quantum dot attached thereto and said first quantum dot is distinguishable from said second quantum dot.

10. (once amended) The method according to claim 1, wherein said first quantum dot is attached to a targeting moiety for said target species, said targeting moiety being a member selected from the group consisting of antibodies,[,] aptamers, proteins, streptavidin, nucleic acids and biotin.

22. (once amended) The method according to claim 19, wherein said alignment moiety [is correlated with] identifies the position of one or more target moiety-affinity complex[es].

25. (once amended) A computer-readable medium encoded with a data set comprising data acquired by [a] the method [according to] of claim 1.

28. (once amended) A computer-readable medium encoded with a database comprising two or more data sets according to claim 25, wherein said database is in a searchable format.

29. (once amended) A method of [detecting] counting a single copy of a target species in solution, said method comprising

(i) detecting a single copy of said target species by detecting essentially simultaneously fluorescence emitted by a first quantum dot of a first color attached to said single copy and a second quantum dot of a second color attached to said single copy, wherein said first color and said second color are distinguishably different colors, and

(ii) resolving said fluorescence emitted by said first quantum dot of a first color attached to said single copy and said second quantum dot of a second color attached to said single copy from fluorescence arising from a quantum dot not attached to said single copy, thereby counting said single copy.

30. (once amended) A method of [detecting] counting a single copy of a target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate with spacing between each member of said population, said method comprising:

(i) detecting a single copy of said target species by detecting fluorescence emitted by a quantum dot attached to said single copy, wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate, wherein said detecting is performed with a detecting means having a resolution that is higher than said spacing between each member of said population, and

(ii) resolving said fluorescence emitted by said quantum dot attached to said single copy from fluorescence arising from a quantum dot not attached to said single copy, thereby counting said single copy.

31. (once amended) A method of [detecting] counting a single copy of a target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate, said method comprising:

(i) detecting a single copy of said target species by detecting fluorescence emitted by a quantum dot attached to said single copy, wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate forming a target-affinity moiety complex, and said detecting is performed with a detecting means having a resolution limited region of interest [such that] whereby, less than one target-affinity moiety complex is present within each resolution limited region of interest, and

(ii) resolving said fluorescence emitted by said quantum dot attached to said single copy from fluorescence arising from a quantum dot not attached to said single copy, thereby counting said single copy.

32. (once amended) A method of [detecting] counting a single copy of a first target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate, said method comprising:

(a) defining a first region of interest of said substrate; and

(b) probing said first region of interest for fluorescence emitted by a quantum dot attached to [a] said single copy of said first target species bound to an affinity moiety for said first target species immobilized on said substrate, wherein said probing resolves said fluorescence from said [first target species] quantum dot from fluorescence arising from other members of said population of target species immobilized on said substrate[.], thereby counting said first target species.

33. (once amended) The method according to claim 32, further comprising [detecting] counting a single copy of a second target species immobilized to said substrate, said method comprising:

(c) defining a second region of interest of said substrate; and

(d) probing said second region of interest for fluorescence emitted by a second quantum dot attached to said [a] single copy of said second target species bound to an affinity moiety for said second target species immobilized on said substrate, wherein said probing resolves said fluorescence from said second [target species] quantum dot from fluorescence arising from other members of said population of target species immobilized on said substrate, thereby counting said second target species.

37. (once amended) A method for [detecting] counting multiple target species immobilized on a substrate, which species are members of a population of target species immobilized on said substrate, said method comprising:

(a) defining multiple regions of interest on said substrate; and

(b) probing said multiple regions of interest for fluorescence emitted by a quantum dot attached to a single copy of said target species bound to an affinity moiety for said target species immobilized within a region of interest of said substrate, wherein said probing resolves fluorescence from said [multiple target species] quantum dot from other members of said population and from each other[.], thereby counting multiple target species.

38. (once amended) A method for determining whether a target species within a region of interest on a substrate is quantifiable by a technique selected from the group consisting of single target counting and ensemble counting, said method comprising:

(a) probing said region of interest to determine target species density within said region of interest by detecting fluorescence emitted by a quantum dot attached to one or more molecules of said target species bound to an affinity moiety for said target species immobilized on said substrate;

(b) comparing said density to a predetermined density cutoff value above which ensemble counting is used and below which single target counting is used[.], thereby determining whether said target species is quantifiable by target counting or ensemble counting.